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Gastric evacuation in the young lemon shark, *Negaprion brevirostris*, under field conditions

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Synopsis

Gastric evacuation in young lemon sharks, *Negaprion brevirostris*, was studied in a field enclosure. Regression analysis was used to evaluate the adequacy of linear, exponential, and square root models in describing the decrease in stomach contents with time after feeding. The linear model produced the best fit and was thus used to compare gastric evacuation at the three temperatures. Gastric evacuation in young lemon sharks is considerably longer than for carnivorous teleosts but shorter than for other elasmobranchs. These differences are the result of differing energy requirements determined by the physiology and behavior of the species.

Introduction

Fisheries management has incorporated the use of multispecies modelling in recent years and examination of trophic relations among fish species represents one of the main tools to express interspecific interactions in a multispecies assessment (Livingston 1986). Determinations of daily ration of the most representative species in an aquatic community are essential to understand their trophic roles in the ecosystem. The most direct approach to estimating daily ration is based on the assessment of amounts of food found in the stomach of fish in the wild. This amount depends on both rates of food intake and of digestion. Therefore, knowledge of the rate of digestion, i.e. gastric evacuation is required to ultimately assess and manage fish stocks.

Gastric evacuation is a flexible process affected by a number of biotic and abiotic variables. Numerous studies have been published on the rate of gastric evacuation in fishes (see Windell 1978, Fänge & Grove 1979, Jobling 1980, Persson 1986 for reviews). However, most of these studies have been conducted in the laboratory and may bear little resemblance to conditions prevailing in nature.

We are aware of only four studies of gastric evacuation in shark species. Two of these, one on the spiny dogfish, *Squalus acanthias* (Jones & Geen 1977), and the other on the sandbar shark, *Carcharhinus plumbeus* (Medved 1985), were completed under field conditions, whereas Schurdak & Gruber (1989) and Macpherson et al. (1989) studied gastric evacuation of the young lemon shark, *Negaprion brevirostris*, and the lesser-spotted dogfish, *Scyliorhinus canicula*, respectively, in the laboratory. Considering their high trophic position, knowledge of a shark's digestion rate is of special importance in assessing its role in the ecosystem.

This study was part of a broader study which investigated the food and daily ration of young lemon sharks (Cortés & Gruber 1990). The purpose of the present experiments was to determine gastric emptying rates of young lemon sharks under field conditions. This study, in conjunction with other studies (Wetherbee et al. 1987, Schurdak & Gruber 1989, Wetherbee & Gruber 1990) was also aimed at improving our understanding of the digestive physiology of sharks.

Materials and methods

A total of 48 juvenile lemon sharks were used to study gastric evacuation in three trials conducted in pen-enclosures at Bimini Lagoon, Bahamas, and Lower Matecumbe, Florida Keys, U.S.A. In trial one (Dec 1985), 8 male and 16 female lemon sharks averaging 52.0 cm (± 3.7 SD) precaudal length (PCL) and 1.520 kg (± 0.480 SD) wet weight were kept in Bimini Lagoon where ambient temperatures ranged from 20 to 25° C. In trial two (Nov 1986), 4 males and 9 females averaging 52.8 cm (± 3.3 SD) PCL and 1.530 kg (± 0.450 SD) weight were also kept at Bimini at temperatures ranging from 21 to 27° C. Trial three (May 1986) was conducted in the Florida Keys at 22.5 to 29° C using 5 males and 6 females averaging 52.8 cm (± 4.5 SD) PCL and 1.570 kg (± 0.360 SD) weight. Pen-enclosures comprised a 20 m square of galvanized wire divided into four equal 10 × 10 m sections. Enclosures were constructed in areas frequented by the sharks and tidal flushing and temperature in the enclosure were the same as those in the surrounding area. Since temperature was uniform throughout the cage, sharks could not modify their temperature behaviorally. Sharks were captured either by dipnetting from an airboat or by hook and line, and transported to the enclosure in less than 5 min. They were then sexed, measured, weighed, and marked for visual identification by punching 4 mm diameter holes in the fins. After allowing 80 h for the fish to evacuate all the food from the gut (Wetherbee et al. 1987) each animal was fed a preweighed meal consisting of a large caudal or head section of fresh snapper, *Lutjanus* spp, or white grunt, *Haemulon plumieri*. These species are common components of the diet of lemon sharks in the study areas (Cortés & Gruber 1990). Meals consisted of a single item, since this appears to be the most common feeding pattern for this species in the field (Cortés & Gruber 1990). The amount of food fed to each shark was 2.7% of its body weight (bw), similar to the ad libitum intake recorded in laboratory experiments (Gruber 1984). The meal was force-fed by pushing the food item into the shark's stomach with forceps. At selected times after feeding sharks were removed from the enclosure, anaesthetized with tricaine (MS-222; 1 : 5000), and stomach contents removed by everting the stomach with forceps (Schurdak & Gruber 1989, Cortés & Gruber 1990). The stomach contents were stored for later analysis in 500 cc polyethylene containers filled with isopropyl alcohol. No sharks regurgitated any meals fed to them or consumed any additional food while in the enclosure. Individual sharks were used up to three times in each experiment; in such cases the sharks were allowed about 48 h to recover from the sampling procedure before resubjecting them to force-feeding.

The recovered stomach contents were dried to constant weight (72 h) at 60° C in a drying oven. The percentage of food remaining in each stomach was determined by dividing dry weight of food remaining by dry weight of the original meal, and multiplying by 100. Dry weight of the meal was obtained from the equation:

$$Y = -0.133 + 0.27\ X\ (r = 0.996),$$

where Y and X are dry and wet weight of the food, respectively. This equation was obtained by regressing wet weight against dry weight for a pooled sample of 25 snappers and grunts. It was estimated that a single regression covered both prey species based on evidence from a previous caloric analysis showing that both snappers and grunts had very similar dry/live weight percentages (Cortés & Gruber 1990).

The evacuation data were fitted to the most widely used mathematical models of gastric evacuation in fishes: linear, exponential, and square root (Jobling 1981, 1986, Persson 1986, Olson & Mullen 1986). The parameters of the linear model were estimated using standard least squares regression. The exponential and square root models were transformed to linear form and then least squares regression performed. The coefficient of variation (CV) of the residuals (Somerton 1980) was chosen as the measure of goodness-of-fit, with CV being expressed as:

$$CV = \frac{\sqrt{\frac{RSS}{N-K}}}{\bar{Y}},$$

where RSS is the residual sum of squares, N is the number of data values, K is the number of parameters in the model, and $\bar{Y}$ is the mean of the dependent variable (percentage of food remaining). This measure of goodness-of-fit has the advantage of being adjusted for the number of parameters in each model and the magnitude of the dependent variable. A smaller value of CV indicates a better fit. The predicted values of Y when $X = 0$, or initial meal size, given by the three mathematical expressions, and an examination of the ANOVA table (Sokal & Rohlf 1981) for each regression were used as additional measures of goodness-of-fit. Finally, the regression lines of the best fitting model for each of the three trials were compared by analysis of covariance (ANCOVA, Sokal & Rohlf 1981) to determine whether there was any evidence of temperature effects on gastric evacuation.

Results

The residuals from the linear, exponential, and square root models fitted to the evacuation data were plotted against time after feeding (X values) for each of the three trials. In all cases there was a systematic pattern caused by unequal variances of the observations (percentage food remaining, Y values). Since one of the assumptions in least squares analysis is that the observations must have equal variances, a weighting factor $w_i = 1/\sigma_i^2$, had to be applied to each data set to equalize the variances of the observations (Draper & Smith 1981). Table 1 summarizes and Figure 1 illustrates the results of the weighted least squares analyses. In trial one, the three models produced very close estimates of the Y-intercept or initial meal size (S_o). The linear model had the lowest coefficient of variation of the residuals ($CV = 0.0083$) but an examination of the ANOVA table revealed that the square root model had the largest proportion of the sum of squares explained by regression ($SS_{reg} =$ 97.3%), suggesting a good fit to the data. In trial two, all models overestimated S_o, with the linear model making the closest prediction (112.1%). The lowest value of CV (0.00477) and the largest SS_{reg} (94.2%) were obtained with the linear model. In trial three, the linear model had the lowest value of CV (0.00706), largest SS_{reg} (99.1%), and closest prediction of S_o (99.3%). The square root model slightly overestimated S_o (103.9%) and also gave a large SS_{reg} (97.9%). The models were also used to calculate the predicted times after feeding required to reach various percentages of food remaining in the stomach (Table 2). In the first stages of evacuation (90 to 50%) the predicted times remained similar among the different models in all three trials. At 25% of food remaining in the stomach, the predictions started to vary considerably between the linear and square root models and the exponential model. These differences increased as the given percentage of food in the stomach decreased (10 to 1%).

Since the linear model produced the best fit overall, it was chosen to compare gastric evacuation under the three temperature regimes. The three linear regressions were compared using analysis of covariance (ANCOVA) and differences in slope were found to be highly significant ($F = 22.59$, 2 and 11 df, $p < 0.001$, $N = 17$). ANCOVA was further computed on paired regressions, and slopes of the regressions from trials 1 and 2 differed significantly ($F = 25.01$, 1 and 8 df, $p < 0.01$, $N = 12$), as did those from trials 1 and 3 ($F = 33.22$, 1 and 8 df, $p < 0.001$, $N = 12$). While the slopes from trials 2 and 3 were not significantly different ($F = 1.32$, 1 and 6 df, $p > 0.05$, $N = 10$), the Y-intercepts from these regressions were highly significantly different ($F = 158.8$, 1 and 7 df, $p < 0.001$, $N = 10$). ANCOVA analyses therefore indicated that temperature affected the rate of gastric emptying.

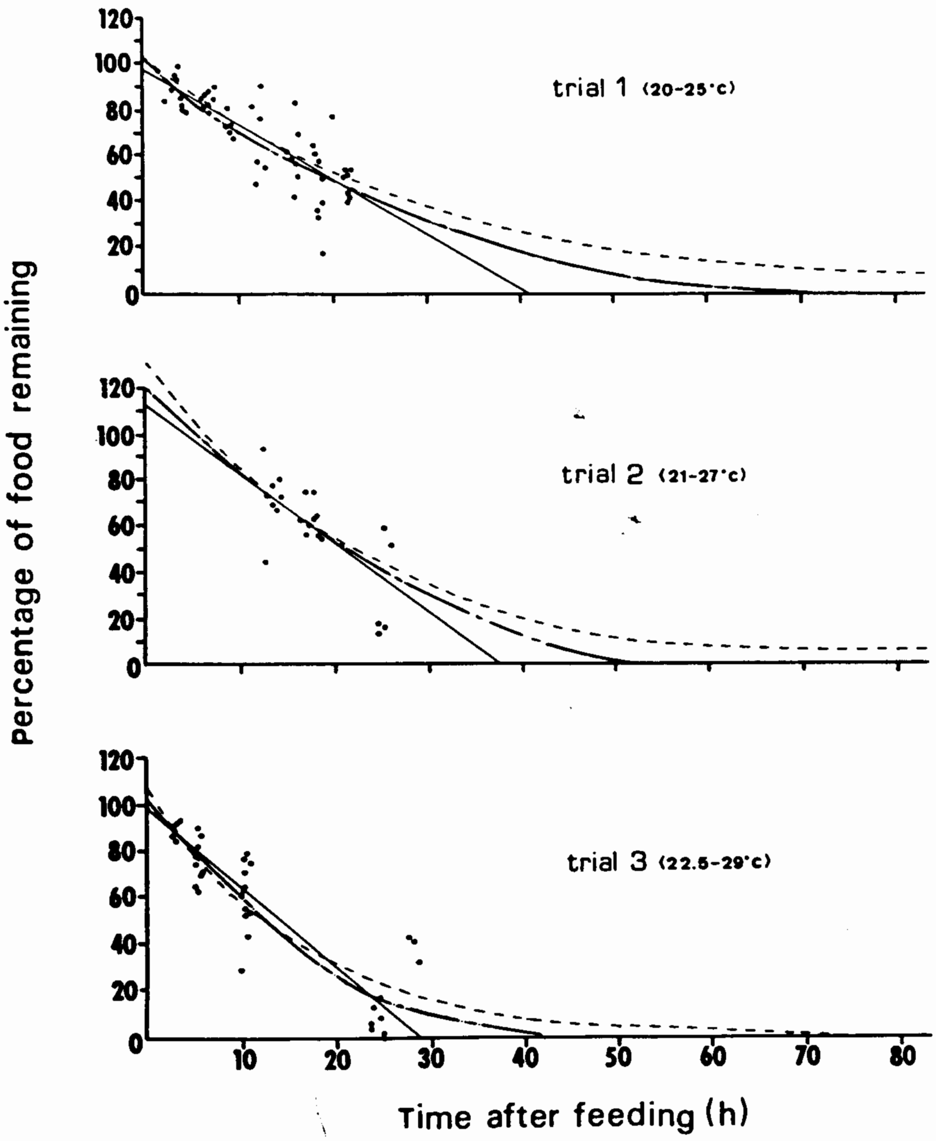

Fig. 1. Mathematical models fitted to gastric evacuation data for young lemon sharks fed snapper or white grunt at three temperature regimes: linear (——), exponential (---) and square root (–··–). Percentage of food remaining in stomach is expressed as dry weight. Equations are given in Table 1.

Discussion

Examination of the coefficients of variation (CV), predicted meals sizes (S_o), and proportion of the sum of squares explained by regression (% SS_{reg}) indicated that the linear model provided the best general description of gastric evacuation for lemon sharks fed snapper or white grunt in the field under the three temperature regimes. After completion of the trials it became apparent that extending food recovery beyond 24–28 h after feeding would have been useful in providing datapoints for the later stages of digestion and might have increased the fit given by the non-linear models. However, the stomachs of three sharks in trial one could not be everted at the planned time after feeding but they did not contain food when everted 48–50 h after being fed. This indicates that even under the lowest

Table 1. Mathematical models fitted to gastric evacuation data for young lemon sharks fed snapper or white grunt at three temperature regimes.

Model	Fitted equation[1]	Y-intercept	CV[2]	% SS_{reg}[3]
Trial 1 (20–25° C)				
Linear	$S_t = 98.5–2.39t$	98.5	$\underline{0.00831}$	91.3
Exponential	$S_t = 102.8e^{-0.03t}$	102.8	0.12739	90.3
Square root	$\sqrt{S_t} = \sqrt{100.6} - 0.141t$	$\underline{100.6}$	0.03479	$\underline{97.3}$
Trial 2 (21–27° C)				
Linear	$S_t = 112.1–3t$	$\underline{112.1}$	$\underline{0.00477}$	$\underline{94.2}$
Exponential	$S_b = 128.5e^{-0.04t}$	128.5	0.08205	88.0
Square root	$\sqrt{S_t} = \sqrt{120.0} - 0.18t$	120.0	0.04270	90.5
Trial 3 (22.5–29° C)				
Linear	$S_t = 99.3–3.46t$	$\underline{99.3}$	$\underline{0.00706}$	$\underline{99.1}$
Exponential	$S_t = 108e^{-0.06t}$	108.0	0.11407	92.1
Square root	$\sqrt{S_t} = \sqrt{103.9} - 0.24t$	103.9	0.04821	97.9

[1] S_t: Amount of food remaining in the stomach at time t; t: time after feeding.
[2] Coefficient of variation of the residuals.
[3] Sum of squares explained by regression of S_t on t, expressed as a percentage of the total sum of squares. 'Best fit' values are underlined.

temperature regime (20 to 25° C), evacuation of a meal was completed in less than 2 d. The linear model predicted that 28.4 to 40.8 h were needed to evacuate 99% of food, whereas predictions by both the square root and exponential models were considerably longer. This gives additional support to the validity of using the linear model to describe gastric emptying in young lemon sharks.

Many variables influence gastric evacuation in fish. Force-feeding may both delay the onset of evacuation and decelerate the rate of emptying when compared to fish fed voluntarily (Windell 1966, Swenson & Smith 1973). In such cases, the best fit should be given by a model incorporating a lag-phase, corresponding to a delay in the onset of gastric emptying. There was no evidence of a lag-phase in the present study, suggesting that any possible stress caused by force-feeding the original meal did not significantly affect evacuation. Extended periods of food deprivation before feeding

Table 2. Time required to reach various stages of gastric evacuation predicted by the models fitted to data for young lemon sharks fed snapper or white grunt at three temperature regimes.

Model	Predicted time (in h) to given percentage of food in stomach						
	90%	75%	50%	25%	10%	5%	1%
Trial 1 (20–25° C)							
Linear	3.6	9.8	20.3	30.8	37.0	39.1	40.8
Exponential	4.4	10.5	24.0	47.1	77.7	100.8	154.4
Square root	3.8	9.7	21.0	35.7	48.7	55.3	64.0
Trial 2 (21–27° C)							
Linear	7.4	12.4	20.7	29.0	34.0	35.7	37.0
Exponential	8.9	13.5	23.6	40.9	63.8	81.2	121.4
Square root	8.2	12.7	21.6	33.1	43.3	48.4	55.3
Trial 3 (22.5–29° C)							
Linear	2.7	7.0	14.2	21.5	25.8	27.2	28.4
Exponential	3.0	6.1	12.8	24.4	39.7	51.2	78.0
Square root	2.9	6.4	13.0	21.6	29.3	33.1	38.3

the experimental subject, or the previous acclimation of the subject can also affect the rate of gastric evacuation (Fänge & Grove 1979). The sharks used in this study were allowed 80 h to completely empty the gut after being captured and were then fed. Since neither captivity nor starvation time exceeded the total food retention time in the alimentary tract (Wetherbee et al. 1987), these two factors are considered to have been of little importance in the current study. Shark size, meal size, and meal type were held constant throughout the trials since the purpose of this study was to obtain a general picture of evacuation for average-size young sharks consuming naturally-occurring prey, rather than details on controlling variables.

Increased temperature has been found to accelerate the rate of digestion in teleosts (Backiel 1971, Kapoor et al. 1975, Persson 1979). Water temperature could not be controlled in this study since sharks were studied in the natural environment and a range of temperatures was recorded for each trial. Nevertheless, ANCOVA analyses indicated that increased temperature accelerated the rate of gastric evacuation in the lemon shark.

The linear model reported in the present study is in apparent contrast to the exponential function fitted to the evacuation data for young lemon sharks by Schurdak & Gruber (1989). Moreover, the time reported by these authors for most sharks to completely evacuate all food from the stomach (24 h) is considerably shorter than the 28.4 to 40.8 h found in the present study. These discrepancies may be explained by the different experimental protocols between the two studies. Firstly, Schurdak & Gruber (1989) fed small, lean, soft, boneless fillets of blue runner, *Caranx chrysos*, to their experimental subjects. This food was more friable and prone to attack by digestive acids and enzymes than the snapper or white grunt sections used in the present study. Since energy content of the meal was very similar in both studies, differing surface-to-volume ratio and friability of the food types may account for the different mathematical models (Jobling 1987). Secondly, in Schurdak & Gruber's (1989) study, the animals fed voluntarily and were allowed at least 2 weeks to recover before being resubjected to the sampling procedure. In the present study, stress associated with force-feeding did not seem to affect the shape of the model. It is hard to assess to which extent allowing our animals only 2 days to recover from the sampling procedure before being resubjected to force-feeding may have affected the evacuation kinetic model.

A review of the literature indicated that emptying of food from the stomachs of lemon sharks (Schurdak & Gruber 1989 and the present study) takes considerably longer than in teleosts. Although gastric evacuation of several teleosts may require over 40 h, the average time for a meal to be completely emptied from the stomach of carnivorous teleosts studied at 20–30° C is less than 19 h (Fänge & Grove 1979). However, gastric evacuation for the lemon shark is faster than in other shark species. Unfortunately, differing methodology and experimental protocols prevent direct comparison among the different species studied. Jones & Geen (1977) reported that digestion of herring took 124 h in spiny dogfish. This long food passage time from the stomach is consistent with the low metabolic rate of this species (Brett & Blackburn 1978) and the low temperatures at which experiments were conducted (10° C). In the lesser-spotted dogfish, the time to evacuate food from the stomach varied according to the type of food and the number of items being fed. Evacuation of 90% of the meal at 14° C was completed in about 30 h for one crustacean item with a thin exoskeleton but evacuation took over 70 h for two crustacean items with thicker, chitinous exoskeletons (Macpherson et al. 1989). Medved (1985) found digestion times of 92.3 and 70.7 h for sandbar sharks fed menhaden and soft blue crab, respectively, at 25.1° C. The long evacuation times for the sandbar shark are reflected in the low daily ration reported for this species (Medved et al. 1988).

In conclusion, gastric evacuation of a 2.7% bw meal takes from 28.4 to 40.8 h at 20–29° C and food is emptied from the stomach in a linear pattern. Gastric emptying in the lemon shark is considerably longer than in carnivorous teleosts, but evacuation is faster than in other sharks. Reduced food intake (Cortés & Gruber 1990) and moderate rate of metabolism (Bushnell 1982) combined with slow rate of digestion and total gut passage time

(Wetherbee et al. 1987) may account for the relatively slow growth of the lemon shark (Gruber & Stout 1983, Gruber 1984) in comparison with carnivorous teleosts.

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